THE EFFECT OF CAFFEINE ON THE BODY WEIGHT OF BALB/C MICE

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ABSTRACT

Objective: To determine the effect of caffeine on the body weight of BALB/c mice.
Study Design: Lab based randomized control trial.
Place and Duration of Study: The study was conducted at Anatomy Department, Army Medical College (AMC), Rawalpindi, in collaboration with National Institute of Health (NIH), Islamabad, from Oct 2014 to Oct 2015.
Material and Methods: Three weeks old BALB/c mice, twenty (20) in number (10 male, 10 female), weighing 12-14 g, were taken and divided into two groups with 10 mice (5 male, 5 female) in each group. The control group G_1 was given standard diet with water *ad libitum*. In addition to the standard diet, the animals in experimental group G_2 were given 10mg of caffeine per 100g body weight once a day on alternate days, three days in a week by oral gavage for 60 days. At the conclusion of the experiment, body weights of the mice in both the groups were measured to determine the influence of caffeine.
Results: The mean final body weight of mice of control group G_1 was observed as 31.2 ± 1.932g while the mean final body weight of experimental group G_2 was found to be 27.1 ± 2.025g. As compared to control group G_1, the final body weight of animals in experimental group G_2 was found to be less.
Conclusion: Ingestion of caffeine has negative effect on the accretion of body weight in mice.

Keywords: Body weight, Caffeine.

INTRODUCTION

Caffeine (1,3,7 trimethylxanthine) is a natural alkaloid found in coffee beans, tea leaves, cocoa beans, cola nuts and other plants. Caffeine is the most frequently ingested pharmacologically active substance in the world[1]. Major intake of caffeine in adults is due to drinking of coffee whereas carbonated soft drinks form primary source of caffeine in children. The main use of caffeine is to enhance alertness and improve short term memory boost specially for the students at college campuses and other recreational spots[2]. Energy drinks often contain additional amounts of caffeine through additives. This global consumption in beverages, foods and numerous pharmaceutical preparations for headache or pain remedies, and allergy drugs, etc. has increased over the years. In this regard, the public and scientific communities have shown interest in the caffeine's potential to produce adverse effects on human health[1].

Numerous researches since 1915 have indicated that ingestion of caffeine provokes an increase in the metabolic rate. However, themetabolic stimulation is dependent on its dosage. Caffeine is also responsible to stimulate thermogenesis and fat oxidation[3]. In animal studies, it was observed that the effect of caffeine was partly due to endogenous catecholamine release and partly to the intrinsic calorigenic mechanism[4]. The caffeine ingestion resulted in an increase in both plasma free fatty acids (FFA) and urinary catecholamine excretion. The FFA response was the outcome of catecholamine induced lipolysis. Caffeine has been shown to increase sympathetic nervous system (SNS) activity, liberating fatty acids from the adipose and/or intramuscular stores[5].

Caffeine was appraised as a thermogenic agent, which in combination with slimming regimens, could be of use in stimulating the loss of body energy[6]. Caffeine can be considered as a non-caloric thermogenic agent that is commonly consumed in many beverages. It has been

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reported that habitual consumption of caffeine equivalent of 1.5 to 1.8g per day in form of coffee could cause mild fever, anorexia, insomnia, and a loss of weight[7]. Although, caffeine is well tolerated by human subjects and no fatalities have been reported as a result of its use; a toxic dose is thought to be about 10g or more[2]. However, caffeine can cause reduction in nutrients absorption rate into the blood stream as well as decreases the rate of fluid absorption or human volunteers by 3-4%[10]. The animal studies have demonstrated that caffeine and other methylxanthines, albeit at high doses, reduced body weight and body fat by both anorectic and thermogenic stimulations[11]. Another recent study has also found that caffeine consumption influenced the energy balance by increasing energy expenditure and decreasing energy intake. Thus causing weight reduction through thermogenesis, fat oxidation, decreased appetite

Table-I: Mean values of the initial and final body weight of animals in control group G_1 and experimental group G_2.

Animal Weight (g)	Group G_1 Mean ± SD (n=10)	Group G_2 Mean ± SD (n=10)	*p*-value
Initial	13.1 ± 0.876	13.1 ± 0.876	1.0
Final	31.2 ± 1.932	27.1 ± 2.025	<0.001

*p-value ≤0.05 is statistically significant.

Table-II: Mean values of the initial and final body weight of male and female animals in control group G_1 and experimental group G_2.

Animal Weight (g)	Group G_1 Male Mean ± SD (n=5)	Group G_1 Female Mean ± SD (n=5)	Group G_2 Male Mean ± SD (n=5)	Group G_2 Female Mean ± SD (n=5)
Initial	13.2 ± 0.836	13.0 ± 1.00	13.2 ± 0.836	13.0 ± 1.00
Final	32.2 ± 1.923	30.2 ± 1.48	28.2 ± 1.643	26.0 ± 1.87

Table-III: Comparison of the final body weight of male and female animals between the groups (control group G_1 and experimental group G_2).

Animal Weight (gm)	Male G_1 vs. Male G_2	Female G_1 vs. Female G_2
	p-value	*p*-value
Final	0.008	0.004

All the p-values are ≤0.05; hence significant.

dehydration during an exercise. Although its excessive dosage provides a sudden discharge of energy to the individual to feel lively initially but this energy is burnt up quickly resulting in reactive hypoglycemia[6].

Caffeine has a direct influence on the basal metabolic rate. A remarkable increase in metabolic rate due to ingestion of caffeine or caffeinated beverages has been reported[8]. There is an increment in metabolic rate as well as in oxidation of lipid. It has been observed that lipid oxidation has been increased in those subjects who performed exercise after caffeine consumption[9]. The single dose oral administration of 100mg caffeine increased the resting metabolic rate of both lean and post-obese and energy intake[12].

Caffeine is consumed in Pakistan in different forms through foods and beverages. However, society is generally unaware due to non-availability of data on its various harmful effects. The present study is an effort towards generating this understanding by gathering information and demonstrating detrimental effect of high caffeine consumption on the body weight of animals (BALB/c mice).

MATERIAL AND METHODS

This laboratory based randomized control trial was carried out in the Department of Anatomy, Army Medical College Rawalpindi, in collaboration with National Institute of Health

(NIH) Islamabad. The study was spanned from October 2014 to October 2015 with the approval of ethical committee on animal experiments, of the Army Medical College, Rawalpindi. The experiment involved three weeks old BALB/c mice having initial weight ranging between 12-14g. The convenience non-probability sampling technique was used. A total of twenty (20) animals, 10 male and 10 female, were selected. given caffeine at a dose of 10mg/100gm bw, on alternate day, 3 days a week for 60 days by oral gavage (fig-1). The body weights of all the animals were recorded at the beginning of the study and also at the conclusion of the study upon completion of 60 days time period. IBM-SPSS version 20 was used for data analysis. The student's t-test was also applied for intergroup comparison of quantitative variable, which was

Figure-1: Photograph showing administration of drug through oral gavage.

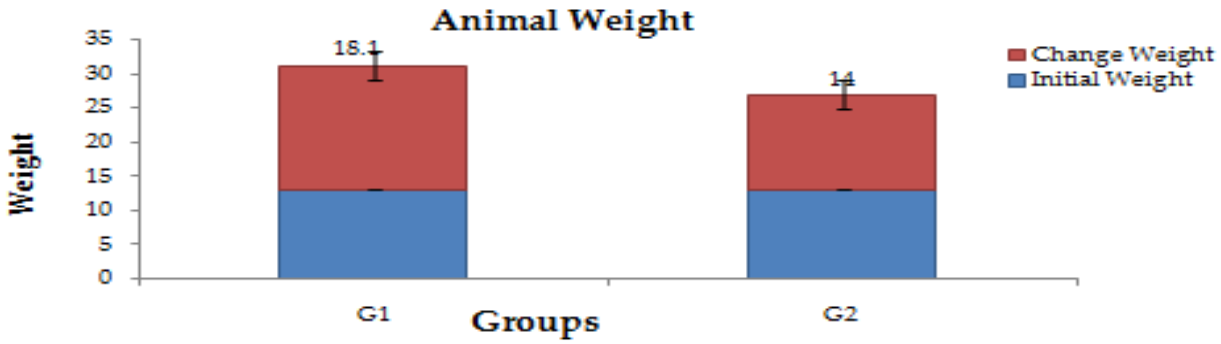

Figure-2: Bar Chart showing comparison of initial and change in mean weights (g) of animals between study groups (control group G_1 and experimental group G_2).

The mice were randomly divided by lottery method into two groups; each group contained 5 male and 5 female mice (10 mice in each group). Laboratory conditions were kept as well ventilated with room temperature range maintained between 20-26°C through environmental control system installed in the laboratory. In order to avoid pregnancy, these male and female mice were kept in separate cages[13]. Standard laboratory diet along with water ad libitum was administered for 60 days to the mice of controls group; this group was titled as G_1. The experimental group (named as G_2) in addition to the standard laboratory diet, were taken as means and standard deviations (mean ± SD). A *p*-value <0.05 was taken as significant.

RESULTS

The total number of BALB/c mice was twenty (20). The mean ± SD initial and final body weights of G_1 animals were observed as 13.1 ± 0.876g and 31.2 ± 1. 932g, respectively. The mean ± SD initial and final body weights of G_2 animals were 13.1 ± 0.876g and 27.1 ± 2.025g, respectively (table-I). The change in final body weight of experimental group G_2 was statistically significant (p<0.05) in comparison with control group G_1 (table-I) (fig-2). The comparison of initial body weights of animals between control

group G_1 and experimental group G_2 was found to be statistically insignificant (table-I). The comparison of body weight of male and female animals between the groups G_1 and G_2 was also found statistically significant (table-III).

DISCUSSION

Caffeine is sold frequently as an ingredient of various food and beverage products[2]. Children and adolescents with eating disorders, especially anorexia nervosa, may regularly consume high amounts of caffeine to counter caloric- restriction associated fatigue, suppress appetite, and produce looser stools and some diuresis[14].

The results show that initial body weight was statistically insignificant between the groups G_1 and G_2 (table-II) as well as on comparison between male and female BALB/c mice of groups G_1 and G_2. On the other hand, the final body weights were found statistically significant when caffeine fed experimental group G_2 was compared with control group G_1 (table-I) (fig-2). Similarly the final body weight was also determined to be significant when comparing male animals of group G_1 with corresponding animals of G_2 and female mice of G_1 group with female mice of G_2 group (table-III).

Although globally caffeine has been an area of interest for the various medical researchers, limited literature of in country studies is available on determining the detrimental effects of caffeine on the animals. The results of current study are comparable with an earlier international study done on male Wristar rats where the final body weight after exposure to caffeine was significantly lower than that of the control group[15]. Yet another study shows that by increasing the catabolism of fatty acid, caffeine causes elevation of the resting metabolic rate and induces a greater loss of body weight[2]. It has been suggested that caffeine ingestion that increased lypolysis, was partially due to increased catecholamine release[4]. Moreover caffeine is involved to liberate the free fatty acids from adipose and intramuscular stores by increasing the activity of sympathetic system[5]. Caffeine reduces body fat by enhancing its oxidation. It escalates lipolysis by inhibiting the cyclic nucleotide phosphodiesterase. In vitro studies have demonstrated that caffeine inhibits phosphodiesterase, the enzyme responsible for degrading cyclic AMP (cAMP). An increase in the cAMP half-life aggrandizing lipolysis, subsequently elevatefatty acid availability for fuel use. Increasing coffee consumption was found to be inversely associated with weight gain[16].

Caffeine is an adenosine receptor antagonist, and studies have suggested that caffeine intake may increase basal energy expenditure and stimulate thermogenesis via the sympathetic nervous system[17]. In the presence of lipolytic hormones, such as adrenaline, caffeine acts synergistically and causes an increase in cyclic AMP concentrations that is greater than that caused by the hormones alone. Hence, it is revealed that caffeine is responsible for promotion of resting energy expenditure, lipid mobilization and fat oxidation[4]. It is demonstrated that caffeine induces inhibitory effects of fat accumulation by upregulating the activities of enzymes involved in hepatic β-oxidation of fatty acid and suppressing the activities of enzymes involved in the fatty acid synthesis. The potential mechanism for suppression of fatty acid synthesis in the liver induced by caffeine is related to its effects on the mRNA expression of lipogenesis-associated genes and subsequent protein synthesis[18]. Caffeine effectively depleted triglyceride and cholesterol levels by inhibition of lipogenesis and stimulation of lipolysis through AMP-activated protein kinase signaling pathway[19].

More research and increased public awareness is needed to bring about a greater understanding of the negative effects of caffeine. The coffee in adults while carbonated soft drinks in children has shown to be the primary source of caffeine[20]. The soft drinks are designed to enhance alertness or provide short term memory boost and are readily available at college campuses and recreational hot spots. Energy drinks often contain additional amounts of

caffeine through additives. Up to 98% of adolescents regularly consume caffeine[21]. Caffeine can result in the slow downing the rate at which nutrient is absorbed into blood stream; it also slows down the rate of fluid absorption or dehydration during an exercise. Excessive caffeine provides a blast of energy enabling the person to feel good initially but when energy is burnt up in 30–400 minutes, there is a sugar crash[22].

CONCLUSION

The present study showsthat high dosage of caffeine has negative effects on the accretion of body weight of the mice.

CONFLICT OF INTEREST

This study has no conflict of interest to declare by any author.

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